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DESCRIPTION AND PHYLOGENETIC RELATIONSHIPS OF TWO NEW SPECIES OF MINIATURE *ARTHROLEPTIS* (ANURA: ARTHROLEPTIDAE) FROM THE EASTERN ARC MOUNTAINS OF TANZANIA

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ABSTRACT. I describe two new species of squeaker frog (Arthroleptidae: *Arthroleptis*) from the Eastern Arc Mountains of Tanzania. These new species are distinguished from other miniature *Arthroleptis* in the Eastern Arc Mountains by the combination of very small adult body size (< 15 mm snout–vent length), a pronounced dark inguinal spot, and color patterns unique to each species. The new species could be the smallest frog species known from East Africa and among the smallest species of *Arthroleptis*. An estimate of phylogeny on the basis of mitochondrial DNA sequences reveals that these morphologically cryptic species are sister taxa that compose a basal lineage within a clade of miniature *Arthroleptis*. Their description hints at a diverse cryptic amphibian fauna in the Eastern Arc Mountains that awaits additional discovery through molecular methods.

KEY WORDS: Africa; biodiversity hotspot; body size; cryptic species; evolution; molecular phylogeny

Ninatoa maelezo hapa kuhusu aina mbili mapya wa vyura (Familia ya Arthroleptidae: kikundi cha *Arthroleptis*) kutoka milima ya 'Tao ya Mashariki' ya Tanzania. Hawa aina mapya ni tofauti na *Arthroleptis* wengine wadogo kwa urefu wa mwili ndogo zaidi (< 15 mm kutoka mapua hadi nyuma ya mwili), doa moja nyeusi na rangi binafsi kwa kila aina mpya. Kwa kutumia sayansi ya DNA inaonyeshwa hawa aina wa vyura wako chini katika kikundi cha *Arthroleptis*. Ugunduzi huu inatoa rai kuhusu aina wengine wa vyura kwa milima ya Tao ya Mashariki wanaongojea kugunduliwa kwa kutumia sayansi ya molekuli.

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The systematics of miniature African squeaker frogs (*Arthroleptis sensu* Blackburn, 2008; Frost, 2008) has been problematic for more than half a century. Historically, many of these small species (< 25 mm snout–vent length [SVL]) were placed in a separate genus, *Schoutedenella* (e.g., Laurent, 1940, 1954, 1973), but most authors have

retained them in *Arthroleptis* (e.g., Perret, 1991; Poynton, 1976, 2003a; Poynton and Broadley, 1985; Schmidt and Inger, 1959; see also Frétey, 2008; Zimkus and Blackburn, 2008). Additional taxonomic problems resulted from disagreement regarding the validity of particular species or genera (e.g., Laurent, 1954, 1961, 1972; Schmidt and Inger, 1959). Recent molecular phylogenetic analysis demonstrated that these miniature species do not form a clade and are paraphyletic with respect to larger *Arthroleptis* (Blackburn, 2008; Frost *et al.*, 2006). Consequently, these two genera were synonymized (Frost *et al.*, 2006). The most recent common ancestor of *Arthroleptis* was miniature, and the evolution of small body size and direct development could have contributed to the dispersal of this lineage from the forests of Central Africa to diverse habitats throughout much of the rest of Africa (Blackburn, 2008). Because many *Arthroleptis* species are small, morphologically similar, or both, many species are probably undescribed and cryptic (e.g., Blackburn, 2008; Poynton, 2003b; Rödel and Bangoura, 2004). Little progress has been made recently in describing the diversity of small *Arthroleptis* species, at least in part because of a lack of genetic resources that could assist in delimiting species boundaries.

A recent phylogenetic study revealed a cryptic lineage of *Arthroleptis* in the Eastern Arc Mountains of Tanzania (Blackburn, 2008). This study investigates this newly discovered lineage, represented by only one specimen in the previous study, through analysis of additional tissue samples and museum specimens of miniature *Arthroleptis* from the Eastern Arc Mountains. This cryptic lineage was found to contain two undescribed species, known only from the northern part of the Eastern Arc Mountains in Tanzania, that could be the smallest frog species both in East Africa and within *Arthroleptis*.

MATERIALS AND METHODS

All measurements (± 0.1 mm) were taken with digital calipers under a dissecting microscope. Measurement methodology follows Blackburn (2005), which is a modification of Matsui (1984). All limb measurements were made on the right side except where indicated. Females were identified by large body size, the presence of ova (visible either in dissection or through the wall), the lack of male secondary sexual characters, or a combination of features; males were identified by the presence of male secondary sexual character typical of *Arthroleptis* (Blackburn, 2009). High-magnification images of specimens were taken by a JVC 3-CCD digital camera mounted on a dissecting microscope with AutoMontage Pro 5.0 (Synoptics). Museum acronyms follow Leviton *et al.* (1985).

Phylogenetic relationships were estimated through analysis of DNA sequences. Data for the mitochondrial 12S and 16S ribosomal RNA (rRNA) genes and the intervening transfer RNA for valine were collected from four specimens. These sequences were added to data collected for *Arthroleptis schubotzi*, *A. xenodactyloides*, and *A. xenodactylus* collected during a recent molecular phylogenetic study (Table 1; Blackburn, 2008). The two new species correspond to taxa that Blackburn and Measey (2009) refer to as *Arthroleptis* sp. nov. A and *Arthroleptis* sp. nov. B. Two distantly related species, *A. stenodactylus* and *A. variabilis*, were used as outgroup taxa (e.g., Blackburn, 2008).

Genomic DNA extracts were made with the use of a Qiagen DNeasy Tissue Kit (Cat. no. 69506). Primer pairs (i.e., 12L1 and 16sh; 12sm and 16sa; 16sc and 16sd) follow Darst and Cannatella (2004); polymerase chain reaction (PCR) and sequencing reactions follow Blackburn (2008). DNA sequences of unequal length ($\sim 1,900$ bp) were aligned in ClustalX v.1.83.1 with default parameters.

TABLE 1. SPECIMENS INCLUDED IN PHYLOGENETIC ANALYSIS.

<i>Arthroleptis</i> sp.	Locality	Coordinates	Catalog No.	GenBank No.	Source
<i>fichika</i> , n. sp.	Baga II Forest Reserve, West Usambara Mountains, Tanzania	04°48'S, 038°27'E	CAS 168829	FJ151064	Blackburn, 2008
	Mazumbai Forest Reserve, West Usambara Mountains, Tanzania	04°49'45"S, 038°30'46"E	MCZ A-138384	FJ188697	This study
cf. <i>fichika</i>	Chome Forest Reserve, South Pare Mountains, Tanzania	04°17'S, 037°55'40"E	FMNH 251864	FJ188696	This study
<i>kidogo</i> , n. sp.	Nguru Mountains, Tanzania	06°03'09"S, 037°32'26"E	MCZ A-138393	FJ188698	This study
			MCZ A-138394	FJ188699–70	This study
<i>schubotzi</i>	Bwindi Impenetrable National Park, Uganda	00°59'34"S, 29°36'57"E	CAS 201752	FJ151061	Blackburn, 2008
			CAS 201753	FJ151116	Blackburn, 2008
<i>xenodactyloides</i>	Amani, East Usambara Mountains, Tanzania	05°05'S, 038°36'E	CAS 168608	FJ151063	Blackburn, 2008
	Ruo River Gorge, Mulanje Massif, Malawi	15°56'S, 035°37'E	MCZ A-137002	FJ151096	Blackburn, 2008
<i>xenodactylus</i>	Amani Nature Reserve, East Usambara Mountains, Tanzania	05°05'37"S, 038°36'00"E	MCZ A-138404	FJ151156	Blackburn, 2008
			MCZ A-138405	FJ151157	Blackburn, 2008
<i>stenodactylus</i>	Amani, East Usambara Mountains, Tanzania	06°56'30"S, 37°43'10"E	CAS 168455	FJ151054	Blackburn, 2008
<i>variabilis</i>	Etome, Petit Mount Cameroon, Cameroon	06°56'30"S, 37°43'10"E	MCZ A-136744	FJ151083	Blackburn, 2008

However, PCR products were amplified and sequenced with varying success. Thus, for two specimens, the full sequence length could not be obtained: a 743 bp fragment of 12S rRNA was obtained from MCZ A-138393; two fragments (12S rRNA, 765 bp; 16S rRNA, 830 bp) were obtained from MCZ A-138394. After alignment, the sequences for the fragments amplified for MCZ A-138394 were merged in MacClade v.4.06 to form a single taxon in the analysis; the intervening base pairs were considered missing data. The alignment was trimmed to positions corresponding to 2,496–4,260 of the *Xenopus laevis* mitochondrial genome (GenBank

NC-001573) such that the final alignment contained 1,902 characters. All but two specimens (MCZ A-138393–4) have no missing data for these 1,902 characters; after trimming, 693 characters are present for all specimens in the analysis.

A maximum likelihood (ML) estimate of phylogeny was obtained through analysis of sequence data in GARLI v.0.95 (Zwickl, 2006) with a random starting tree and a GTR+I+Γ model of evolution with all parameters estimated. The GARLI analysis was terminated 250,000 generations after the last topological improvement. Support for phylogenetic topologies was estimated by

nonparametric bootstrapping in GARLI. One thousand bootstrap replicates were performed with the same model of evolution, with each search terminated 1,000 generations after the last topological improvement. Branches present in $\geq 70\%$ of the bootstrap trees were considered well supported following Hillis and Bull (1993). In addition, a Bayesian estimate of phylogeny was obtained with MrBayes v.3.1.1 and a GTR+I+ Γ model of evolution. Bayesian analysis was run for 5 million generations, sampled every 1,000 generations, with four chains, a temperature of 0.2, and default priors. The first 1 million generations were discarded as burn-in. The phylogeny and posterior probabilities were then estimated from the 4,000 post-burn-in trees. Topologies with posterior probabilities $\geq 95\%$ were considered well supported (Wilcox *et al.*, 2002). Genetic distances were calculated in MEGA v.4.0.1 (Tamura *et al.*, 2007) with the maximum composite likelihood (MCL). The MCL calculations were performed with the use of data for transitions and transversions and assuming a heterogeneous pattern of sequence evolution with a Γ distribution of among site rate variation (Γ parameter = 1.0).

DESCRIPTION OF NEW SPECIES

Arthroleptis fichika, new species

Hidden Squeaker Frog

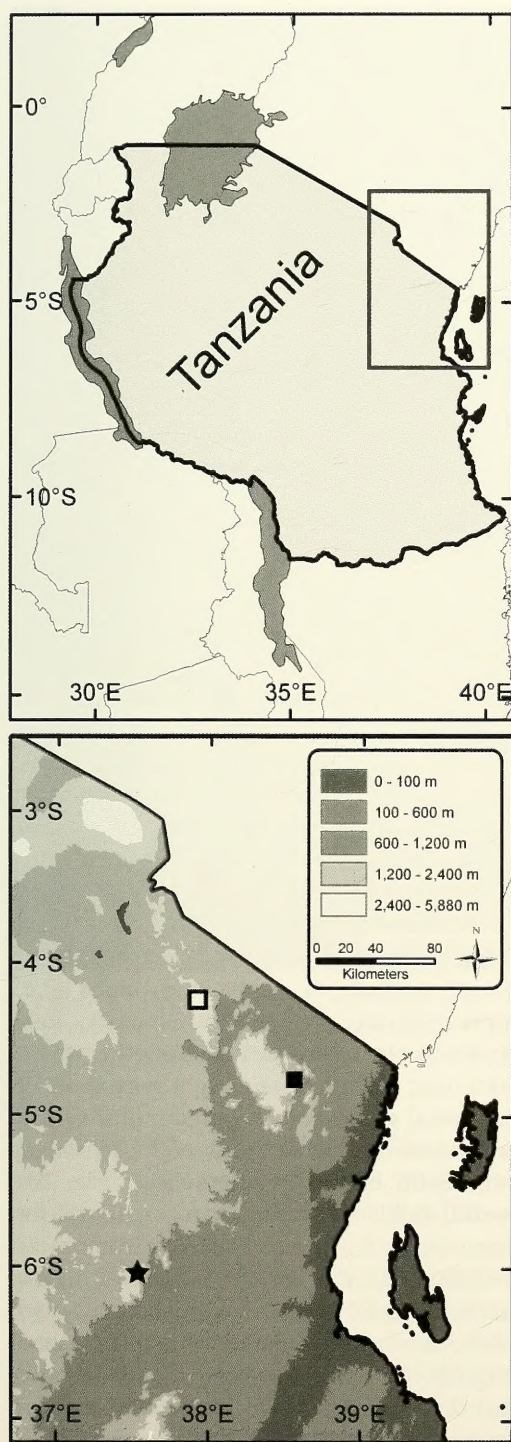
Figures 1–4; Table 2

Holotype. MCZ A-138384 (field no. Breda M. Zimkus [BMZ] 23104), adult female (gravid), Republic of Tanzania, Tanga Region, West Usambara Mountains, Mazumbai Forest Reserve, 04°49'45"S, 038°30'46"E (WGS datum), 1,383 m elevation, 10 March 2007, D. L. Mahler, L. Lawson, B. M. Zimkus.

Paratype. CAS 168829 (field no. Robert C. Drewes [RCD] 11039), adult female, Republic of Tanzania, Tanga Region, Lushoto District, West Usambara Mountains,

Baga II Forest Reserve, 04°48'S, 038°27'E (estimated), 1,500–1,900 m elevation (estimated from latitude and longitude), 26 April 1988, R. C. Drewes, K. M. Howell, and J. V. Vindum.

Diagnosis. A miniature *Arthroleptis* similar to other small East African *Arthroleptis* (i.e., *A. schubotzi*, *A. stridens*, *A. xenochirus*, *A. xenodactyloides*, *A. xenodactylus*, and the new *Arthroleptis* species described below), but distinguished from all but the other new species by smaller adult body size (gravid adult females < 15 mm SVL; Table 3) and a prominent dark brown inguinal spot that is darker than other prominent dorsal markings and the dorsal base color. An inguinal spot is variably present in other miniature *Arthroleptis* from East Africa, but it is neither as prominent nor as dark relative to other dorsal markings as it is in these two new species. *Arthroleptis fichika* differs from the new species described below by less expanded and less pointed toe tips, a supratympanic band that terminates anterior to the arm (Fig. 4), a prominent dark brown spot on the anterior distal thigh, and lacking a reticulated pattern on the ventral surface of the head and body. *Arthroleptis fichika* differs in the following additional ways from other miniature East African *Arthroleptis*: from *A. schubotzi*, *A. stridens*, and *A. xenochirus* by darkly colored ventral thighs with small light gray spots; from *A. schubotzi*, *A. stridens*, and *A. xenodactyloides* by a less globular and more elongate inner metatarsal tubercle; from *A. stridens* by less expanded toe tips, more defined dorsal and lateral markings, and more ventral pigmentation; from *A. xenochirus* by the crus longer than the thigh, lacking accessory metatarsal tubercles at the base of the second and third toes, and more expanded and pointed digit tips; from *A. xenodactylus* by more defined dorsal markings and less expanded and less pointed toe tips.



Description of Holotype. Very small (SVL 13.5 mm) gravid female; limbs relatively slender (Figs. 2–4; Table 2); head broad; head length 94% head width; snout barely projecting beyond lower jaw (Fig. 4A); rostral tip slightly rounded and nearly straight in dorsal view, nearly straight in lateral view; eyes projecting just beyond margins of head in dorsal view; dorsal surface of eyes approximately level with dorsal surface of head in lateral view; eye diameter just barely wider than interorbital distance; pupils small, horizontal, and elliptical in preservative; loreal region nearly flat; naris rounded and directed laterally with ventral margin visible in dorsal view; canthus rostralis short, slightly convex; eye diameter 2.4 times eye–narial distance; eye diameter nearly 10 times distance from naris to rostral tip; internarial region slightly convex; internarial distance 84% interorbital distance; tympanum rounded, height less than half diameter of eye; tympanic annulus well defined; supratympanic region smooth with no fold; tongue large and posteriorly expanded with narrow anterior attachment; tongue with prominent rounded posterior notch; tongue lacking median papilla; choana completely hidden behind maxillary shelf in ventral view; premaxillary and maxillary teeth present but hidden in labial view by lips; vomerine teeth absent.

Skin of limbs and dorsal, lateral, and ventral surfaces of head and body smooth; skin ventral to cloaca very weakly tuberculate; median skin raphe only barely visible in preservative; cloacal opening horizontal.

Figure 1. Type localities of *Arthroleptis fichika* n. sp. (black square) and *A. kidogo* n. sp. (black star) in the mountains of eastern Tanzania. Locality of specimen referred to *A. fichika* (FMNH 251864) is indicated by an open square.

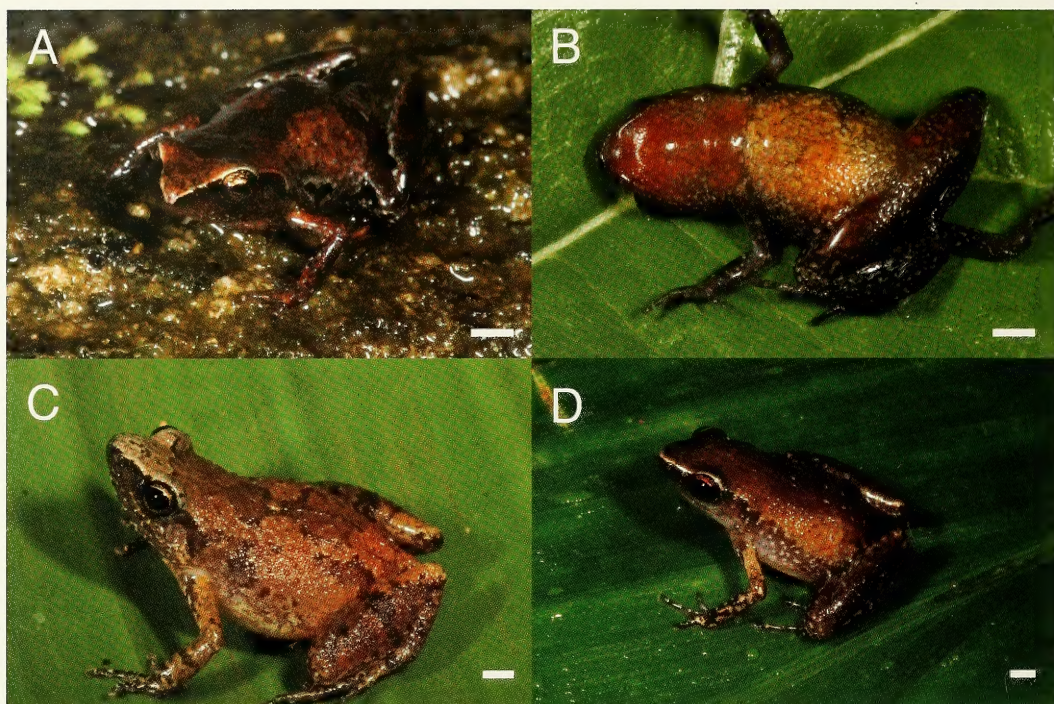


Figure 2. A, B, *Arthroleptis fichika* n. sp. (holotype, MCZ A-138384); C, *Arthroleptis xenodactyloides* (MCZ A-138386); and D, *Arthroleptis xenodactylus* (MCZ A-138401) in life. Scale bars ~ 2 mm. Photographs by D. L. Mahler.

Limbs and digits slender; relative length of fingers: $\text{III} > \text{II} > \text{IV} > \text{I}$; finger tips neither swollen nor expanded; palmar and metacarpal tubercles very weakly developed and completely flat; webbing between manual digits absent; fingers with somewhat distinct, weakly globular, and single subarticular tubercles; thigh length 88% crus length; relative length of toes: $\text{IV} > \text{III} > \text{V} > \text{II} > \text{I}$; toe tips weakly pointed, slightly expanded, and just wider than interphalangeal regions; webbing between pedal digits absent; toes with indistinct, single subarticular tubercles; inner metatarsal tubercle small, indistinct, and elongate, length 57% of first toe length; outer metatarsal tubercle absent.

Measurements. See Table 2.

Coloration in Life. From photographs of holotype. Dorsal base coloration ruddy brown with darker brown markings

(Fig. 2A); iris golden and vermiculated with black; pupil black; light gray to cream thin line extending from snout tip through margin of upper eyelid; lateral surface of body with dark gray base coloration; markings on lateral surface of body and limbs (i.e., supratympanic, inguinal, femoral) dark brown to black and darker than other dorsal markings; scattered small light gray spots on the lateral surface of body and limbs; ventral surface of head (i.e., gular region) orangey red; venter bright creamy yellow (Fig. 2B); ventral thigh with distinct red base coloration.

Coloration of Holotype (in Alcohol). Dorsal base color light brownish gray with scattered small darker brown spots (Fig. 3A); iris dark brown with silver pupil; dark brown interorbital bar incomplete and broken into chain of small spots; snout

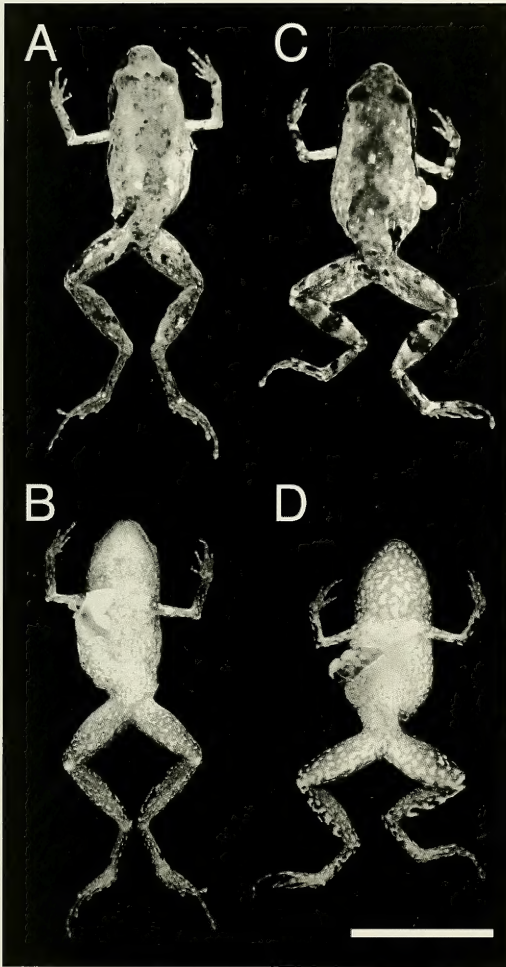


Figure 3. Holotypes in dorsal and ventral views. A, B, *Arthroleptis fichika* n. sp. (MCZ-138384); C, D, *Arthroleptis kidogo* n. sp. (MCZ A-138394). Scale bar = 10 mm.

darker gray than more posterior head; loreal and suborbital regions medium brown but not forming a mask; continuous dark brown supratympanic band extending from posterior margin of eye, over tympanum, and terminating well anterior to forelimb (Fig. 4A); tympanum brown, translucent, and distinct from supratympanic band; three poorly defined, but interconnected, gray-brown spots arranged along dorsal midline between head

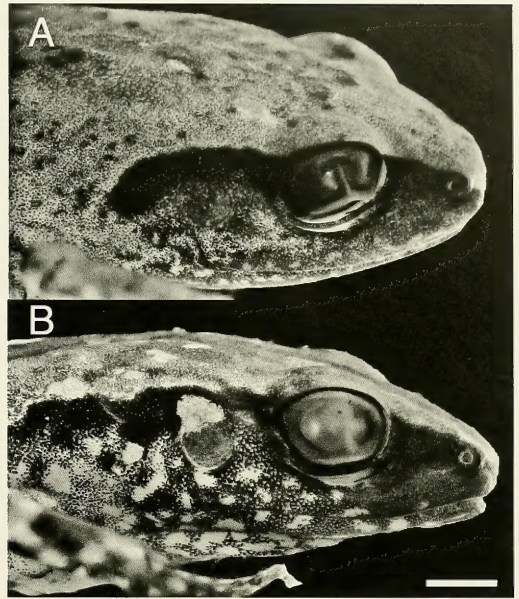


Figure 4. Right lateral view of heads of holotypes. A, *Arthroleptis fichika* n. sp. (MCZ-138384); B, *Arthroleptis kidogo* n. sp. (MCZ A-138394). Scale bar = 1 mm.

and sacrum (i.e., typical “hour-glass” configuration of *Arthroleptis*); margins of dorsal spots defined by irregularly spaced small dark brown spots; lateral surface of body with several dark brown spots between forelimb and hind limb; prominent, dark brown inguinal spot extending from base of hind limb over the lateral surface of the ilium (Fig. 3A); dorsal surfaces of limbs similar light gray, grading to darker gray on distal hind limbs; scattered small dark brown spots on the dorsal surfaces of forelimb; ultimate interphalangeal joints unpigmented and light gray in color; dark brown spot on posterodorsal surface of thigh; proximal anterior surface of thigh with small light gray spots; prominent dark brown spot on anterior surface of distal thigh; hind limbs distal to knee covered in brownish light and dark gray mottling; dark brown trapezoid centered on cloaca.

Gular region and venter with cream base color and small dark brown melanocytes

TABLE 2. MEASUREMENTS (MM) OF *ARTHROLEPTIS FICHIKA* AND *A. KIDOGO*.

	<i>A. fichika</i>		<i>A. kidogo</i>	
	MCZ A-138384	CAS 168829	MCZ A-138394	MCZ A-138393
	Holotype	Paratype	Holotype	Paratype
Snout-vent length	13.5	14.2	14.1	13.9
Head width	4.8	5.0	5.0	5.1
Tympanum height	0.8	0.8	0.8	0.8
Eye diameter	1.9	1.9	1.7	2.1
Snout length	1.2	1.5	1.4	1.5
Forearm length	3.2	3.0	2.9	2.9
Manual digit I	0.7	0.7	0.7	0.8
Manual digit II	0.9	1.1	1.0	1.0
Manual digit III	1.5	1.9	1.6	1.5
Manual digit IV	0.8	0.9	0.8	0.8
Thigh length	5.7	6.2	6.3	6.0
Crus length	6.5	6.5	6.3	6.3
Pedal digit I	0.7 (left)	0.7	0.8	0.9
Pedal digit II	1.3	1.3	1.3	1.3
Pedal digit III	1.9	2.2	2.1	1.9
Pedal digit IV	2.9	3.3	3.2	2.8
Pedal digit V	1.6	1.4	1.7	1.4
Inner metatarsal tubercle	0.3	0.4	0.5	0.5

forming no distinct pattern (Fig. 3B); ventral forelimbs dark brown with irregularly sized and poorly defined small light gray spots; palmar and plantar surfaces dark gray brown; ventral hind limb dark brown with many small light gray spots at somewhat regular intervals.

Variation. The dorsal surface of the paratype (CAS 168829) has a continuous wide brown band extending from between the eyes to just anterior to the cloaca. Light brown regions border this midline band laterally. The prominent dark spot in the inguinal region and anterior surface of distal thigh are readily apparent in both the paratype and the referred specimen.

Habitat and Natural History. The holotype (MCZ A-138384) was collected during a daytime visual survey (0800–1100 h) when it was active in leaf litter in dense forest (from field notes of BMZ).

Conservation. The two collection localities are both in the forests of the West Usambara

Mountains, which have an estimated extent of less than 320 km² (Burgess *et al.*, 2007) and are threatened by forest loss and degradation associated with population growth (Kaoneka and Solberg, 1994). Because *A. fichika* presently appears to be restricted to these forests, this new species should be considered tentatively as Vulnerable according to IUCN (2001) criteria.

Etymology. The specific epithet *fichika* should be treated as an indeclinable word. It is a Kiswahili word meaning “hidden” and refers to the fact that *A. fichika* is a cryptic species first identified through molecular phylogenetic analysis (Blackburn, 2008).

Phylogenetic Relationships. See below.

***Arthroleptis kidogo*, new species**

Tiny Squeaker Frog

Figures 1, 3, 4; Table 2

Holotype. MCZ A-138394 (BMZ 23288), adult female (gravid), Republic of Tanzania, Nguru South Forest Reserve, Morogoro

TABLE 3. MEAN SNOUT-VENT LENGTH (SVL) AND STANDARD DEVIATION OF MINIATURE *ARTHROLEPTIS* FROM KENYA, MALAWI, TANZANIA, AND UGANDA.

<i>Arthroleptis</i> sp.	Locality	Mean SVL (mm)	
		Female	Male
<i>fichika</i>	West Usambara Mtns., Tanzania	13.9 ± 0.5 n = 2	
<i>kidogo</i>	Nguru Mtns., Tanzania	14.0 ± 0.1 n = 2	
<i>schubotzi</i>	Bwindi Forest, Uganda ¹	19.1 ± 1.5 n = 4	19.6 ± 0.9 n = 6
<i>stridens</i>	East Usambara Mtns., Tanzania ²		17.9 n = 1
<i>xenochirus</i>	Zambezi River Source, Zambia ³	20.5 n = 1	18.5 ± 1.6 n = 9
<i>xenodactyloides</i>	Taita Hills, Kenya ⁴	19.6 ± 2.0 n = 4	17.3 n = 1
	West Usambara Mtns., Tanzania ⁵	18.9 ± 2.2 n = 5	
	East Usambara Mtns., Tanzania ⁶	20.3 ± 2.1 n = 6	
	Misuku Hills, Malawi ⁷	16.7 ± 0.7 n = 4	12.8 ± 1.1 n = 2
	Mulanje Massif, Malawi ⁸	16.5 ± 0.8 n = 16	15.2 ± 0.2 n = 3
	Mt. Chelinda, Zimbabwe ⁹	17.1 ± 0.8 n = 22	14.5 ± 1.0 n = 15
<i>xenodactylus</i>	East Usambara Mtns., Tanzania ¹⁰	17.5 ± 1.3 n = 10	13.7 ± 0.1 n = 2
	Nguru Mtns., Tanzania ¹¹	17.0 n = 1	14.2 ± 0.8 n = 3

¹CAS 104500-01, 201700, 201717-19, 201736-39

²ZMB 66249

³CAS 196614, 196617-18, 196620-21, 196623, 196627, 196630, 196632, 196638

⁴NMK A/4538, A/4540, A/4542, A/4653/1-2

⁵MCZ A-138385-89

⁶CAS 168608, FMNH 251405, MCZ A-13199, A-138390-92

⁷MCZ A-137136-41

⁸MCZ A-137001-15, A-137034-37, A-137074

⁹MCZ A-17038, A-23339-50, A-19047-67; TMP 19101, 19104.

¹⁰MCZ A-13188, A-13190-94, A-13196, A-138404-05, A-138429, A-138435, A-138437

¹¹MCZ A-138400-03

Region, Mvomero District, Nguru Mountains, 06°03'09"S, 037°32'26"E (WGS datum), 830 m elevation, 31 March 2007, D. L. Mahler and B. M. Zimkus.

Paratype. MCZ A-139393 (BMZ 23287), adult female (gravid), same collection data as holotype.

Diagnosis. A miniature *Arthroleptis* similar to other small East African *Arthroleptis* (i.e., *A. fichika*, *A. schubotzi*, *A. stridens*, *A. xenochirus*, *A. xenodactyloides*, *A. xenodactylus*) but distinguished by a prominent dark brown supratympanic band that continues posterior to the level of the arm (Fig. 4B). In

other species, the supratympanic band terminates anterior to or at the level of the arm (Figs. 3C, D, 4A). *Arthroleptis kidogo* is differentiated from all Eastern Arc *Arthroleptis* except *A. fichika* by smaller adult body size (gravid adult females < 15 mm SVL; Table 3) and a prominent dark brown inguinal spot that is darker than other prominent dorsal markings and the dorsal base color. *Arthroleptis kidogo* differs in the following additional ways from other miniature *Arthroleptis* in East Africa: from *A. fichika* by dark anterior thighs with many small light gray spots and a reticulated pattern of dark melanocytes and light gray spots on the ventral surface of the head and body; from *A. schubotzi*, *A. stridens*, and *A. xenochirus* by darkly colored ventral thighs with small light gray spots; from *A. schubotzi*, *A. stridens*, and *A. xenodactyloides* by a less globular and more elongate inner metatarsal tubercle and expanded digit tips with distinctly pointed toe tips; from *A. xenochirus* by a crus that is longer than the thigh, lacking accessory metatarsal tubercles at the base of the second and third toes, and much more expanded and pointed digit tips; from *A. xenodactylus* by more defined dorsal markings and digit tips that, although pointed, do not exhibit a papillate projection.

Description of Holotype. Very small (SVL 14.1 mm) gravid female; limbs relatively slender (Figs. 3, 4; Table 2); head broad; head length 90% head width; snout barely projecting beyond lower jaw; rostral tip only slightly rounded in dorsal view, nearly straight in lateral view (Fig. 4B); eyes projecting just beyond margins of head in dorsal view and just above dorsal surface of head in lateral view; eye diameter approximately equal to interorbital distance; pupils horizontally elliptical in preservative; loreal region nearly flat; naris rounded and directed laterally, not visible in dorsal view; canthus rostralis short, slightly convex; eye diameter

1.5 times eye-narial distance; eye diameter 5.6 times distance from naris to rostral tip; internarial region slightly convex; internarial distance 83% interorbital distance; tympanum rounded, height slightly less than half diameter of eye; tympanic annulus well defined; supratympanic region smooth with no fold; tongue narrow and ovoid with minute posterior notch; tongue lacking median papilla; choana just barely visible behind maxillary shelf in ventral view; premaxillary and maxillary teeth present but hidden in labial view by lips; vomerine teeth absent.

Skin of limbs and dorsal and lateral surfaces of head and body smooth; ventral surface of head and body smooth anteriorly, but very weakly tuberculate posteriorly; median skin raphe absent, at least following preservation; cloacal opening horizontal, surrounded by smooth skin.

Limbs and digits slender; relative length of fingers: $\text{III} > \text{II} > \text{IV} \geq \text{I}$; finger tips not swollen or expanded but distinctly pointed; palmar and metacarpal tubercles present but weakly developed and flat; webbing between manual digits absent; fingers with somewhat indistinct, flat, and single subarticular tubercles; thigh length approximately equal to crus length; relative length of toes: $\text{IV} > \text{III} > \text{V} > \text{II} > \text{I}$; toe tips expanded to approximately twice the width of interphalangeal regions; webbing between pedal digits absent; each toe tip with prominent narrow distal point; toes with prominent, single, flattened subarticular tubercles; inner metatarsal tubercle small, indistinct, and elongate, length 62% of first toe length; outer metatarsal tubercle absent.

Measurements. See Table 2.

Coloration in Life. No photographs or field notes are available to document the coloration of *A. kidogo* in life.

Coloration of Holotype (in Alcohol). Dorsal base color light gray (Fig. 3C); iris dark

gray to black with silvery pupil; snout and midline dorsal markings medium brown; dorsal markings extremely poorly defined, but three confluent brown spots apparent along dorsal midline extending from posterior head to sacrum (i.e., typical “hour-glass” configuration of *Arthroleptis*); loreal and suborbital regions medium brown forming near continuous mask; mask broken posteriorly by small light gray spots between eye and tympanum; continuous dark brown supratympanic band extending from posterior margin of eye, over tympanum, and terminating posterior to arm on lateral surface of body (Fig. 4B); tympanum light brown, translucent, and grading into supratympanic band; lateral surface of body light gray with scattered, irregular unpigmented spots; prominent, dark inguinal spot extending laterally over ilium and terminating over the sacroiliac joint; dorsal surfaces of forelimbs light gray; dark brown band on the dorsal surfaces of forearm; ultimate interphalangeal joints unpigmented and light gray in color; dorsal surfaces of hind limbs brown with irregular light gray spots; anterior surface of thigh dark brown with well-defined, small, light gray spots; crus with prominent transverse dark brown band; feet medium to dark brown dorsally with scattered gray spots; poorly delimited dark brown circle centered on cloaca with pair of darker brown spots at anterodorsal margin.

Gular region with reticulated pattern of small dark brown melanocytes and many light unpigmented spots (Fig. 3D); reticulated pattern extending on ventral surface of proximal forelimb and terminating posteriorly at level of pectoral girdle; venter mostly unpigmented and creamy gray; some scattered small melanocytes forming poorly defined reticulated pattern at lateral and more posterior margins of ventral surface; palmar and plantar surfaces dark brown

with scattered small unpigmented spots; ventral hind limb dark brown with many small light gray spots.

Variation. The paratype is very similar to the holotype with the following exceptions: margins of the dorsal markings are highlighted in places by thin, dark spots; mask is unbroken posteriorly with no small gray spots between eye and tympanum; reticulations on the ventral head and legs are lighter and slightly less defined; subarticular tubercles on the pedal digits are slightly more globular.

Habitat and Natural History. Both specimens were collected during daytime visual surveys (1300–1600 h) when they were active in leaf litter adjacent to a trail running through the forest.

Conservation. The sole locality lies in the forests of the Nguru Mountains, which have an estimated extent of < 300 km² (Burgess *et al.*, 2007) and are threatened by forest loss and degradation (Menegon *et al.*, 2008). Because *A. kidogo* is only known from these forests, this new species should be considered tentatively as Vulnerable according to IUCN (2001) criteria.

Etymology. The specific epithet *kidogo* should be treated as an indeclinable word. It is the Kiswahili word meaning “very small” in recognition that this is among the smallest species of *Arthroleptis*.

Remarks. The diagnosable color patterns of adult *A. fichika* and *A. kidogo* are similar to those of juveniles of other *Arthroleptis* species in the Eastern Arc Mountains. For example, juvenile specimens probably referable to *A. xenodactyloides* (MCZ A-139017–21) have two prominent features: a dark inguinal spot and a dark lateral bar extending from the snout tip, over the eye and tympanum, and terminating on the posterior lateral surface of the body wall, sometimes extending nearly into the inguinal region. Thus, caution is needed in identifying spec-

imens. *Arthroleptis fichika* and *A. kidogo* are clearly distinct evolutionary lineages (see *Phylogenetic Relationships* below), and the adults are morphologically distinguishable from adults of other *Arthroleptis* in the Eastern Arc Mountains. Because the juvenile color patterns of these and other larger *Arthroleptis* are both poorly documented and likely very similar, molecular data may be required to identify small juvenile specimens.

Phylogenetic Relationships. The ML estimate of phylogeny is well supported by both nonparametric bootstrap values and Bayesian posterior probabilities (Fig. 5). *Arthroleptis fichika* and *A. kidogo* are divergent (mean: 13.0%) and sister species. These two new species form a clade sister to a clade containing *A. xenodactylus*, *A. xenodactyloides*, and *A. schubotzi* (Fig. 5; Blackburn, 2008). All of the species in this clade are found in the mountains of East Africa; *A. xenodactyloides* and *A. xenodactylus* are sometimes also found at lower elevations (Channing and Howell, 2006). *Arthroleptis xenodactyloides* and *A. xenodactylus* are syntopic at some localities (e.g., Amani Forest Reserve, East Usambara Mountains, Tanzania), but, as found by Blackburn (2008), these species are not sister taxa. Instead, *A. xenodactyloides* is the sister to *A. schubotzi*, which is restricted to the Albertine Rift Mountains of Burundi, Rwanda, Uganda, and eastern Democratic Republic of Congo where *A. xenodactyloides* is not known to occur (IUCN, 2008). The phylogenetic affinity of *A. xenochirus* remains unknown because of a lack of genetic resources.

DISCUSSION

Because these new taxa are morphologically distinguishable and evolutionarily divergent, recognition of two species is war-

ranted. Differences in color pattern clearly differentiate the type specimens of *Arthroleptis fichika* and *A. kidogo*. Furthermore, the pairwise divergence between *A. fichika* and *A. kidogo* is on par with that between other species such as *A. schubotzi* and *A. xenodactyloides* (mean: 16.7%), *A. schubotzi* and *A. xenodactylus* (mean: 17.4%), and *A. xenodactyloides* and *A. xenodactylus* (mean: 12.1%).

This phylogenetic analysis also included an additional gravid female specimen (FMNH 251864) collected in a pitfall trap in Chome Forest Reserve (04°17'S, 037°55'40"E [datum unavailable], 2,000 m) in the South Pare Mountains, just to the northwest of the West Usambara Mountains. Although morphologically similar to *A. fichika*, the sequence data for FMNH 251864 demonstrates that the specimen is divergent (16.7%) from the two type specimens. Indeed, if this specimen is considered conspecific with *A. fichika*, then this would be the greatest amount of intraspecific sequence divergence so far observed for this genetic locus in *Arthroleptis* (Blackburn, 2008). Thus, this specimen is only tentatively designated as *A. cf. fichika* and clearly could represent yet another miniature cryptic species. Future field research focused on collecting additional specimens is needed to evaluate further the taxonomic status of miniature *Arthroleptis* in the poorly studied South Pare Mountains.

The Eastern Arc Mountains of Tanzania and Kenya constitute a global hotspot of biodiversity (Burgess *et al.*, 2007; Myers *et al.*, 2000). The climate of these mountains is under direct influence of the climatic regime in the Indian Ocean (Marchant *et al.*, 2006) and is believed to have been relatively stable since the uplift of these mountains approximately 30 million years ago (Burgess *et al.*, 2004). It is unclear whether the high levels of endemic biodiversity in these mountains are

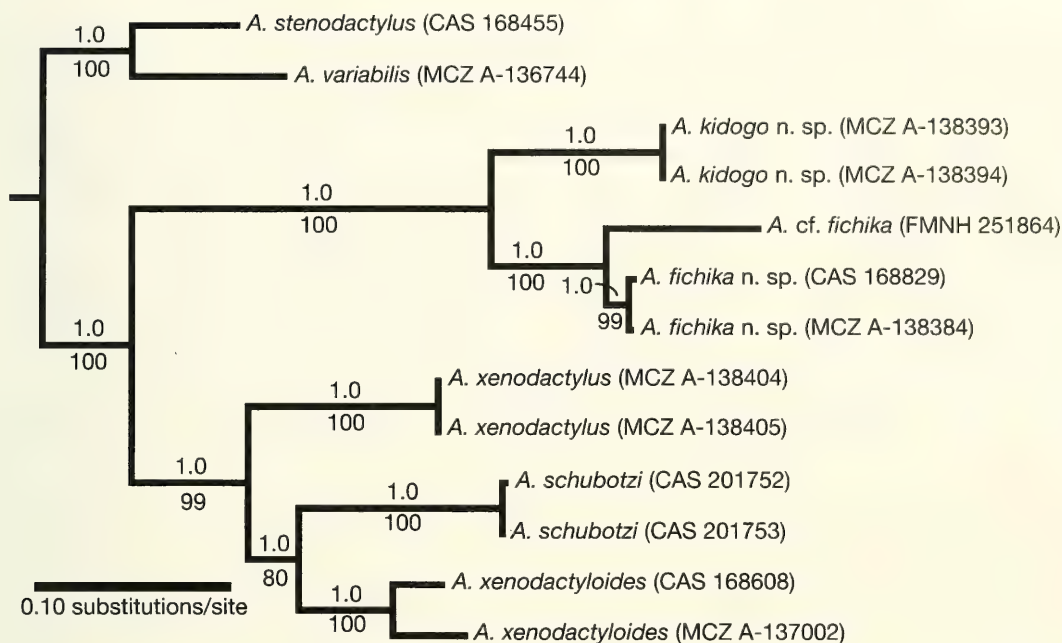


Figure 5. Phylogram estimated from mitochondrial DNA sequences depicting relationships of miniature *Arthroleptis* in the Eastern Arc Mountains. Numbers above branches are Bayesian posterior probabilities and below branches are nonparametric bootstrap proportions.

the result of higher speciation rates, lower extinction rates, or some combination of both (Lovett *et al.*, 2005). Biodiversity research has been carried out in these mountains for more than a century, but both cryptic and surprisingly distinct vertebrate taxa continue to be described (e.g., Channing and Stanley, 2002; Davenport *et al.*, 2006; Fjelds  *et al.*, 2006). The number of new amphibian species recently described from the Eastern Arc Mountains is truly remarkable, and this trend shows no sign of abating (e.g., Channing and Stanley, 2002; Loader *et al.*, 2006; Menegon *et al.*, 2004, 2007; M ller *et al.*, 2005; Pickersgill, 2007; Poynton, 2003b; de S  *et al.*, 2004). These descriptions contribute to making this region one of the hotspots of global amphibian diversity (Stuart *et al.*, 2004). However, to date, molecular phylogenetic study has played a relatively small role in describing

this diversity. Undoubtedly, many more cryptic amphibian species are yet to be described from East Africa and molecular analysis will play an important role in this work.

Recently, Pickersgill (2007) described *Arthroleptis stridens* from Kambai and Longuza Forest Reserves in the East Usambara Mountains. The taxonomic status of *A. stridens* is difficult to evaluate because molecular data are unavailable, an audio-spectrogram was not published, it is known from only one adult specimen (the holotype), and morphological characters that differentiate this species from the very similar *A. xenodactylus* were not presented. Pickersgill (2007) differentiated *A. stridens* from *A. xenodactylus* by its call, but did not compare it to the call of *A. xenodactylus*. My study of the holotype of *A. stridens* indicates that, although morphologically sim-

ilar to these two species, *A. stridens* can be differentiated from *A. xenodactyloides* and *A. xenodactylus* by a greater degree of sexual dimorphism. Males of many *Arthroleptis* species have third fingers that are relatively longer than those of conspecific females (Blackburn, 2009). Notably, *A. xenodactyloides* and *A. xenodactylus* are two of the few *Arthroleptis* species with little sexual dimorphism in third finger length (Blackburn, 2009). The holotype of *A. stridens* (ZMB 66249) is a male and has a notably elongate third finger (21.8% SVL) compared with *A. xenodactyloides* (mean from type locality, Mt. Selinda, Zimbabwe: 16.7% SVL; $n = 16$) and *A. xenodactylus* (mean from Nguru Mountains, Tanzania: 12.7% SVL; $n = 3$). Relative male third finger length is much greater in two other East African miniature *Arthroleptis*, *A. schubotzi* (mean from Bwindi Impenetrable Forest, Uganda, 30.8%; $n = 6$) and *A. xenochirus* (mean from Zambezi River Source, Zambia, 27.5%; $n = 9$), but these species do not co-occur with *A. stridens*. Additional specimens are needed to confirm whether relatively longer male third fingers is diagnostic of *A. stridens* relative to *A. xenodactylus* and *A. xenodactyloides*. More generally, specimens with associated tissue samples and recorded calls are critically needed in the study of African frog diversity. Without these data, African frog systematics will continue to be plagued by potentially unresolvable taxonomic problems, such as the status of *Arthroleptis stridens*.

The diversity of miniature *Arthroleptis* species remains poorly described and little studied. Because of the morphological similarities among these species, molecular data can be used to recognize species boundaries and thus enable better morphological diagnoses. The two new *Arthroleptis* species described here might be both the smallest *Arthroleptis* species (e.g., Blackburn, 2008; Laurent, 1954) and the smallest frog species

in Tanzania (e.g., Channing and Howell, 2006). The maximum snout–vent length of gravid females of both *A. fichika* and *A. kidogo* is < 15 mm. Males of either species are currently unknown. However, because mean male body size is less than that of females for all known *Arthroleptis*, it is probable that males, when found, will also be smaller than 15 mm SVL. It is possible that these species have been overlooked previously because their small size is similar to that of small juveniles of other *Arthroleptis*. Furthermore, it is likely that other undescribed miniature *Arthroleptis* species from East Africa are present in existing museum collections but are misidentified as juveniles of larger species or confused with other miniature species. Maximum and mean snout–vent length of *A. fichika* and *A. kidogo* are clearly less than those of other miniature *Arthroleptis* in East Africa (Table 3; *Material Examined*). Interestingly, northern populations of the widespread *Arthroleptis xenodactyloides* are larger than southern populations. A recent investigation of body size evolution in *Arthroleptis* found that body size has both decreased and increased several times across the phylogeny (Blackburn, 2008). For instance, the two largest *Arthroleptis* species, both from the Eastern Arc Mountains (*A. tanneri*, Grandison, 1983; *A. nikeae*, Poynton, 2003b), are not closely related, which indicates that large body size has been attained at least twice independently (Blackburn, 2008). The diversity of cryptic miniature and large *Arthroleptis* within East Africa awaits further molecular and anatomical study.

MATERIAL EXAMINED

Type specimens and reference samples, sex, and snout–vent length (in mm) indicated in parentheses.

Arthroleptis schubotzi. Burundi: ZMB 21774 (holotype; female, 20.4); Uganda: CAS 104500–01 (females, 17.2, 19.3), 201700

(female, 18.8), 201717 (male, 19.2), 201718 (female, 20.9), 201719 (male, 18.5), 201736–39 (males, 20.0, 19.6, 18.9, 21.1).

Arthroleptis stridens. Tanzania: ZMB 66249 (holotype; male, 17.9).

Arthroleptis xenochirus. Angola: BMNH 1947.2.30.54 (holotype; male, 17.8); Democratic Republic of Congo: MCZ A-21794 (male, 20.8), A-21799 (female, 20.7); Zambia: CAS 196614 (male, 17.9), 196617–18 (males, 19.0, 18.8), 196620–21 (males, 18.1, 15.9), 196623 (male, 19.0), 196627 (male, 16.9), 196630 (male, 20.8), 196632 (male, 20.5), 196638 (female, 20.5), MCZ A-37418 (female, 19.8).

Arthroleptis xenodactyloides. Kenya: NMK A/4653/1 (female, 15.5), A/4538 (female, 20.5), A/4542 (female, 20.7), A/4540 (female, 20.6), A/4653/2 (female, 16.7); Malawi: MCZ A-137001 (male, 15.4), A-137002 (female, 15.5), A-137003 (male, 15.3), A-137004 (female, 16.8), A-137006–13 (females, 18.0, 16.8, 16.4, 15.8, 15.8, 16.9, 16.7, 14.8), A-137014 (male, 15.0), A-137015 (female, 16.2), A-137034–37 (females, 16.5, 17.6, 15.7, 17.3), A-137074 (female, 17.0), A-137136–37 (females, 17.4, 17.3), A-137138–39 (males, 12.0, 13.5), A-137140–41 (females, 16.2, 16.0), TMP 84805 (female, 16.7); Tanzania: CAS 168608 (female, 23.4), FMNH 251405 (female, 18.2), MCZ A-13199 (female, 18.2), A-13210 (juvenile, 14.1), A-25403 (female, 19.2), A-25404–05 (juveniles, 13.9, 12.9), A-138383 (female, 16.6), A-138385–92 (females, 19.8, 19.4, 14.9, 20.1, 20.2, 19.6, 22.2, 20.3); Zimbabwe: A-17038 (female, 16.3), A-19038 (female, 17.4), A-139047 (female, 17.3), A-139048–49 (females, 16.3, 16.3), A-139050 (female, 18.0), A-139051 (female, 16.8), A-139052 (female, 16.5), A-139053 (female, 17.5), A-139054 (male, 16.1), A-139055–56 (female, 18.1, 15.9), A-139057 (female, 16.4), A-139058 (female, 16.8), A-139059 (female, 16.5), A-139060–61 (males, 14.3, 16.5), A-139062–63 (females, 16.1, 16.9), A-139064 (female, 17.1), A-139065 (female,

17.7), A-139066–67 (females, 17.2, 16.9), A-23339–50 (males, 14.4, 13.9, 14.4, 14.0, 14.5, 13.1, 14.5, 13.7, 15.7, 13.6, 14.8, 13.7), TMP 19101 (female, 18.4), 19104 (female, 19.0).

Arthroleptis xenodactylus. Tanzania: BMNH 1947.2.6.92 (holotype; unknown, 15.3), MCZ A-138188 (female, 17.5), A-13190 (male, 13.8), A-13191–94 (females, 18.0, 16.0, 19.6, 15.7), A-13196 (male, 13.6), A-13400 (male, 14.5), A-138401 (female, 17.0), A-138402–03 (males, 14.8, 13.3), A-138404–05 (females, 17.4, 16.5), A-138435 (female, 17.1), A-138437 (female, 19.2), A-138429 (female, 18.3).

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